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Comparative analysis of intraoperative liquid biopsy in tumor margin detection during hepatobiliary and gastrointestinal cancer surgeries

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Abstract:
Accurate intraoperative tumor margin assessment remains a major challenge in hepatobiliary and gastrointestinal cancer surgery, leading to residual disease and increased recurrence risk. Therefore, it is of interest to evaluate the diagnostic performance of intraoperative liquid biopsy (ctDNA, CTCs and exosomes) compared with frozen section histopathology in 90 patients undergoing oncological resection. The combined liquid biopsy panel achieved 93.4% sensitivity, 89.7% specificity and 90.3% concordance with R0 margin status, outperforming frozen section analysis in sensitivity. Liquid biopsy also demonstrated a lower re-excision rate and a shorter turnaround time, supporting its feasibility for real-time intraoperative decision-making. Thus, data shows the multi-analyte intraoperative liquid biopsy may enhance margin assessment accuracy and represent a promising adjunct to conventional frozen section evaluation.

Keywords: Intraoperative liquid biopsy, circulating tumor DNA (ctDNA), tumor margin detection, hepatobiliary surgery, gastrointestinal cancer, circulating tumor cells (CTCs)

Background:
Hepatobiliary and gastrointestinal malignancies, including hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), colorectal cancer (CRC) and gastric cancer (GC), are major contributors to cancer-related morbidity and mortality worldwide. Surgical resection remains the cornerstone of curative treatment, with achievement of histologically negative margins (R0 resection) being a critical determinant of recurrence-free survival and long-term prognosis **[1, 2]**. However, accurate intraoperative assessment of tumor margins remains challenging despite advances in imaging and surgical techniques. Positive or close margins continue to occur in a substantial proportion of cases, increasing the risk of local recurrence and adversely affecting patient outcomes **[3]**. Frozen section histopathology is currently the standard method for intraoperative margin assessment. Although widely used, it is limited by sampling errors, tissue-processing artifacts, dependence on expert pathological interpretation and prolonged turnaround times that may interrupt surgical workflow **[4]**. These limitations are particularly relevant in hepatobiliary and minimally invasive gastrointestinal procedures, where complex anatomy and restricted tissue access can compromise margin evaluation **[5]**. Consequently, residual positive margin rates remain clinically significant, highlighting the need for more accurate and timely intraoperative assessment strategies **[6]**. Liquid biopsy has emerged as a promising minimally invasive approach for detecting tumor-derived biomarkers, including circulating tumor DNA (ctDNA), circulating tumor cells (CTCs) and extracellular vesicles**.** Unlike conventional tissue sampling, liquid biopsy can capture tumor heterogeneity and provide real-time molecular information from multiple tumor sites **[7]**. Recent technological advances in digital droplet PCR, next-generation sequencing and microfluidic platforms have improved assay sensitivity and reduced analytical turnaround times, making intraoperative application increasingly feasible **[8]**. Preliminary studies suggest that intraoperative changes in ctDNA levels may correlate with completeness of tumor resection and residual disease status**.** However, comprehensive comparisons between intraoperative liquid biopsy and frozen section histopathology across hepatobiliary and gastrointestinal malignancies remain limited **[9].** Therefore, it is of interest to report the diagnostic performance and clinical utility of intraoperative liquid biopsy for tumor margin assessment compared with conventional frozen section histopathology in hepatobiliary and gastrointestinal cancer surgery.

Methodology:
This prospective comparative observational study was conducted at a tertiary care oncology center over a period of 18 months. A total of 90 adult patients scheduled to undergo curative-intent surgical resection for histologically confirmed hepatobiliary or gastrointestinal malignancies were enrolled after written informed consent was obtained. The study protocol was approved by the Institutional Ethics Committee in accordance with the ethical principles of the Declaration of Helsinki (2013 revision). The study included patients with primary hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), colorectal cancer (CRC), or gastric cancer (GC), aged 18 years or older, with resectable disease as determined by a multidisciplinary tumor board and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. Exclusion criteria encompassed patients with unresectable or metastatic disease at time of surgery, concurrent hematological malignancies, active systemic infection, prior participation in an investigational drug trial within 30 days, inability to provide informed consent, or inadequate blood volume for liquid biopsy sampling. Patients receiving anticoagulation therapy at the time of surgery were also excluded to minimize hemolytic interference with ctDNA extraction. Participants were divided into two disease groups for subgroup analysis: Group A (hepatobiliary cancers: HCC and CCA, n=45) and Group B (gastrointestinal cancers: CRC and GC, n=45). Intraoperative blood samples (10 mL each) were collected at three standardized time points: prior to surgical incision (baseline), immediately following tumor mobilization and within five minutes of tumor specimen removal. Plasma was isolated by double centrifugation

and stored at −80°C pending analysis. ctDNA was extracted using a validated commercial cfDNA extraction kit and quantified by digital droplet PCR (ddPCR) targeting tumor-specific somatic mutations identified from pre-operative tissue biopsy sequencing. CTCs were isolated using a microfluidic-based immunomagnetic separation platform employing anti-EpCAM antibodies, followed by cytokeratin immunofluorescence staining and enumeration. Tumor-derived exosomes were isolated by differential ultracentrifugation and characterized by nanoparticle tracking analysis and surface protein profiling. A combined liquid biopsy panel integrating all three analytes was constructed for composite analysis. Frozen section histopathology was performed contemporaneously on designated margin specimens submitted by the operating surgeon according to standard protocol, with results reported by a dedicated onco-pathologist blinded to the liquid biopsy findings. The primary outcome was the diagnostic accuracy of each liquid biopsy analyte and the combined panel expressed as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for detection of positive surgical margins, using permanent section histopathology as the gold standard reference. Secondary outcomes included turnaround time (TAT), re-excision rate and false negative rate. Statistical analysis was performed using SPSS version 26.0; continuous variables were compared by the independent-samples t-test, categorical variables by the chi-square test and diagnostic accuracy parameters by receiver operating characteristic (ROC) curve analysis. A p-value <0.05 was considered statistically significant.

Results:

A total of 90 patients were enrolled, comprising 45 with hepatobiliary cancers (HCC: n=24, CCA: n=21) and 45 with gastrointestinal cancers (CRC: n=25, GC: n=20). The mean age of the cohort was 59.8 ± 9.8 years, with 55 males (61.1%) and 35 females (38.9%). Tumor stage III or IV was present in 60% of the overall cohort. Baseline demographic and clinicopathological characteristics were well balanced between the two disease groups, with no statistically significant differences in age, sex, tumor stage, or neoadjuvant therapy receipt, as shown in **Table 1**. The diagnostic accuracy parameters for each intraoperative liquid biopsy analyte and the combined panel, compared against permanent section histopathology as the reference standard, are presented in **Table 2**. The combined liquid biopsy panel demonstrated the highest sensitivity (93.4%) and specificity (89.7%), with a PPV of 91.2% and NPV of 92.1%, outperforming all individual analytes. ctDNA achieved the highest single-analyte sensitivity (87.6%), while CTCs demonstrated the highest single-analyte specificity (84.8%). Intraoperative frozen section histopathology recorded a sensitivity of 89.2% and specificity of 91.4%. The combined panel sensitivity was significantly superior to frozen section alone (p=0.038). The concordance between intraoperative liquid biopsy and the final margin status on permanent histopathology, stratified by cancer subtype, is summarized in **Table 3**. Overall R0 concordance across all cancer types was 90.3%. Colorectal cancer demonstrated the highest R0 concordance (93.6%) and R1 detection rate (90.1%), while gastric cancer showed the lowest concordance (87.9%) with a false-negative rate of 12.1%. All concordance values were statistically significant compared to chance (p<0.05). **Table 4** presents the comparison of turnaround time, re-excision rate and missed positive margins across the liquid biopsy strategy, frozen section alone, combined approach and intraoperative palpation. The intraoperative liquid biopsy (ctDNA) demonstrated a mean TAT of 28.4 ± 6.2 minutes and a re-excision rate of 7.8%. The combined liquid biopsy plus frozen section approach achieved the lowest re-excision rate (4.4%) and missed margin rate (5.6%), albeit with a longer TAT. Standard intraoperative palpation was associated with the highest re-excision (18.9%) and missed positive margin rates (21.3%), both significantly inferior to liquid biopsy (p<0.001).

Table 1: Baseline demographic and clinicopathological characteristics of study participants

Characteristic	HCC / CCA (n=45)	CRC / GC (n=45)
Age (years, mean ± SD)	58.4 ± 9.6	61.2 ± 10.1
Male / Female	28 / 17	27 / 18
Tumor Stage (III/IV, %)	62.2%	57.8%
Neoadjuvant Therapy (%)	33.3%	40.0%
Median Tumor Size (cm)	4.8 ± 1.6	4.2 ± 1.4
CEA / AFP elevated (%)	AFP: 68.9%	CEA: 71.1%

Table 2: Diagnostic accuracy of intraoperative liquid biopsy analytes versus frozen section histopathology for tumor margin detection

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ctDNA (cfDNA)	87.6	82.4	84.1	86.3
CTCs	79.3	84.8	81.6	83.0
Tumor-derived Exosomes	82.1	80.6	79.4	83.8
Combined Panel	93.4	89.7	91.2	92.1
Intraoperative Histopathology	89.2	91.4	90.3	90.6

Table 3: Concordance of intraoperative liquid biopsy with final margin status stratified by cancer subtype

Cancer Type	R0 Concordance (%)	R1 Detection (%)	False Negative (%)	p-value
Hepatocellular Carcinoma	91.3	86.7	8.7	<0.001
Cholangiocarcinoma	88.4	83.2	11.6	0.003
Colorectal Cancer	93.6	90.1	6.4	<0.001
Gastric Cancer	87.9	82.6	12.1	0.006
Overall	90.3	85.7	9.7	<0.001

Table 4: Comparison of turnaround time, re-excision rate and missed positive margins across intraoperative assessment methods

Method	Mean TAT (min)	Re-excision Rate (%)	Positive Margin Missed (%)	p-value
Intraoperative Liquid Biopsy (ctDNA)	28.4 ± 6.2	7.8	9.7	<0.001
Frozen Section Histopathology	34.6 ± 7.8	12.3	10.8	Reference
Combined LB + Frozen Section	38.2 ± 8.1	4.4	5.6	0.004

Standard Intraoperative Palpation	N/A	18.9	21.3	<0.001

Discussion:

This study provides a comprehensive prospective comparative evaluation of intraoperative liquid biopsy accuracy for tumor margin detection in hepatobiliary and gastrointestinal cancer surgeries. The combined liquid biopsy panel achieved a sensitivity of 93.4% and specificity of 89.7%, with an overall R0 concordance of 90.3%, supporting its clinical viability as a real-time adjunct to conventional margin assessment in oncological surgery. Kalil *et al.* (2024**) [10]** conducted a landmark prospective cohort study in patients undergoing hepatic resection for CRC liver metastases and reported that intraoperative ctDNA clearance correlated significantly with R0 resection status, with a sensitivity of 88.4% for detecting residual disease at surgical margins closely mirroring our ctDNA sensitivity of 87.6% for hepatobiliary cancers. Their study further demonstrated that persistent ctDNA elevation following specimen removal was independently associated with early hepatic recurrence, underscoring the prognostic relevance of intraoperative molecular monitoring that our concordance data also support **[11]**. In contrast, Wang *et al.* (2025) **[12]** reported substantially lower ctDNA sensitivity (72.8%) for intraoperative margin detection in cholangiocarcinoma, which they attributed to the low tumor cellularity and high stromal content characteristic of CCA an observation consistent with our finding that CCA demonstrated the second-lowest R0 concordance (88.4%) and highest false-negative rate (11.6%) among hepatobiliary cancers, suggesting that CCA remains a challenging substrate for ctDNA-based margin assessment. Padillo-Ruiz *et al.* (2024) **[13]** compared intraoperative liquid biopsy with frozen section histopathology in pancreatic and biliary tract surgery and reported a mean liquid biopsy TAT of 31.2 minutes, comparable to our figure of 28.4 minutes, while noting that frozen section TAT of 38.6 minutes significantly extended operative time. Their study documented a re-excision rate reduction from 14.2% with frozen section alone to 6.1% with liquid biopsy-guided surgery a finding broadly consistent with our observed re-excision rates of 12.3% versus 7.8% respectively. Furthermore, Samol *et al.* (2024) **[14]** demonstrated in a multicenter prospective trial that a combined liquid biopsy plus frozen section strategy reduced missed positive margin rates to 5.4% compared to 10.9% for frozen section alone in colorectal liver metastasis surgery, aligning with our combined approach result of 5.6% and supporting the complementary value of integrating both modalities rather than replacing one with the other. The convergence of our results with published literature reinforces that intraoperative liquid biopsy represents a reproducible, minimally invasive and clinically meaningful adjunct for margin assessment across multiple hepatobiliary and gastrointestinal cancer subtypes.

Conclusion:

We show the combined liquid biopsy panel provided accurate and rapid intraoperative assessment of tumor margins across hepatobiliary and gastrointestinal malignancies. Its high concordance with R0 resection status and lower re-excision rates highlight its potential clinical value. This study advances current evidence by supporting multi-analyte liquid biopsy as a feasible adjunct to conventional margin assessment techniques.

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